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The CO_2 Method to Control Insect Infestation in Tree Seed

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DANIDA FOREST SEED CENTRE

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CONTENTS

1. INTRODUCTION

This Technical Note describes the use of CO_2 for the control of insect infestation in tropical tree seed as an alternative to insecticides.

Most relevant literature is concerned with agricultural seed, and information on hard-coated seed of dry-zone woody species is limited.

This note is based partly on literature, partly on experience and an experiment on dry-zone woody species of Burkina Faso.

The study was conducted in Burkina Faso by Danida Forest Seed Centre (DFSC), DanishPest Infestation Laboratory (DPIL) and Centre National de Semences Forestieres (CNSF), Burkina Faso, and was concerned with the effects of various non-poisonous methods including CO_2 on wild populations of insects in the hard-coated seed of *Acacia* and *Bauhinia* species.

Promising results have been reached, but experience with the CO_2 method for tree seed is still limited.

There are several reasons for looking at alternative methods for insect control in tree seed. Firstly, insect infestation causes great losses of seed of many important tropical tree species; insect control is generally attempted by insecticides, many of which pose a health hazard to humans and the environment as a whole. Secondly, tree seed is being transferred between countries on an increasing scale, with a concurrently increasing risk of transferring pests; quarantine stations may treat seed with poisonous chemicals to kill insects. This may reduce the viability of the seed; and the subsequent handling of the poison-covered seeds in the laboratory and the nursery constitutes a health hazard.

A number of non-poisonous treatments are harmful to insects: low relative humidity, high CO_2 concentration, low oxygen concentration, low temperature, high temperature, mechanical impact and radiation. Apart from the CO_2 method, heating and freezing seem to be simple, cheap and effective possibilities, but proper dosages and techniques still have to be developed. Combinations of methods may prove highly effective since smaller dosage is required at high stress levels. Additional research into this area is considered highly relevant.

2. FACTORS INFLUENCING THE CO_2 METHOD

Modified atmospheres are usally either nitrogen enriched, oxygen deficient or CO_2 enriched. Much work has been done to apply these methods to control insects, especially to those on grain and foodstuff.

It has been reported that a CO_2 atmosphere has a significant negative effect on all insects at any developmentl stage. CO_2 does not only act by displacing oxygen, but the exact way of inducing insect damage by CO_2 is however, not known (Annis 1986, Bailey & Banks 1980).

The success of the CO_2 method depends on the following factors:

- composition of the atmosphere (i.e. concentration of CO_2 and oxygen)
- time of exposure
- temperature
- air humidity
- insect species
- developmental stage of the insects (eggs, larvae, pupae and adults)
- stress factors

2.1. Composition of the Atmosphere

Normally, when CO_2 concentration is below 40%, the effect on mortality of adult insects, lavae and pupae is reduced. However, in some studies of 4 insect species, 99.9% mortality was seen within 20 days' exposure at only 20% CO_2 (20^{o}C) (Bailey & Banks 1980). Even 4%, and probably even lower concentrations, can be completely lethal if other influencing factors are present (see for example air humidity, below).

It has been reported that the following combinations of 16, 22 or 34 days of controlled carbon dioxide concentrations of 80%, 60% and 40% respectively can kill even the most resistant species (Annis 1986). It is DFSC's experience that the treatment should last for 8 weeks at 28^{o}C, initially at not less than 60% CO_2 concentration.

The concentration of CO_2 will change, especially in the first 24 hours, because of adsorption in the seed (Mitsuda *et al.* 1973). During the first few days, the concentration from bottom to top may vary (highest at bottom if the bag is upright). It will then stabilize to an overall common level from where it will decline at a rate depending on the CO_2 permeability of the container material (Annis & Graver 1985). The laminated plastic bags mentioned in the appendix of this note have so far kept their low internal pressure for 6 years. The pressure in bags containing *Pinus oocarpa, Acacia tortilis* and *Acacia nilotica* was still only 40-75% of the atmosphere pressure after more than 3 years' storage at 4^{o}C.

Concentrations of oxygen below approx. 5% increase mortality while higher oxygen concentrations reduce it (Annis 1986). However, the effect of the oxygen concentration strongly depends on insect species. When the bag is filled to a CO_2 concentration of 60%, the oxygen concentration is, as expected, reduced to 40% of the initial concentration [i.e. from 21% (of normal atmosphere) to 8%].

2.2 Time of Exposure

Before a treatment schedule can be determined, the relationship between exposure time and mortality must be clarified. For a number of insect species infesting grain and foodstuffs, it has been shown that exposure time of more than 5 days at a CO_2 concentration of 40% and above will lead to the death of more than 50% of the insects. After 2 weeks the mortality exceeds 95% (Annis 1986). Annis (1986) reviewed the literature and concluded that (1) a CO_2 concentration of 40% for 17 days, (2) a CO_2 concentration of 60% for 11 days or (3) a CO_2 concentration of 80% for 8.5 days was effective for most insect species.

In 1990, an experiment with a number of CO_2 treatments was carried out at Centre National de Semences Forestieres, Burkina Faso, on wild populations of insects (bruchids) feeding on hard-coated seed of *Acacia nilotica, A. dudgeoni, A. seyal, A. senegal, A. albida* and *Bauhinia rufescens* trees (the method is described in section 4).

Some results are shown in figure 1. It is seen that seed viability was not affected. It also shows that most of the effect on insect reduction is achieved already in the first week. For two out of eight seed lots the depletion was complete (Mourier & Sary 1990).

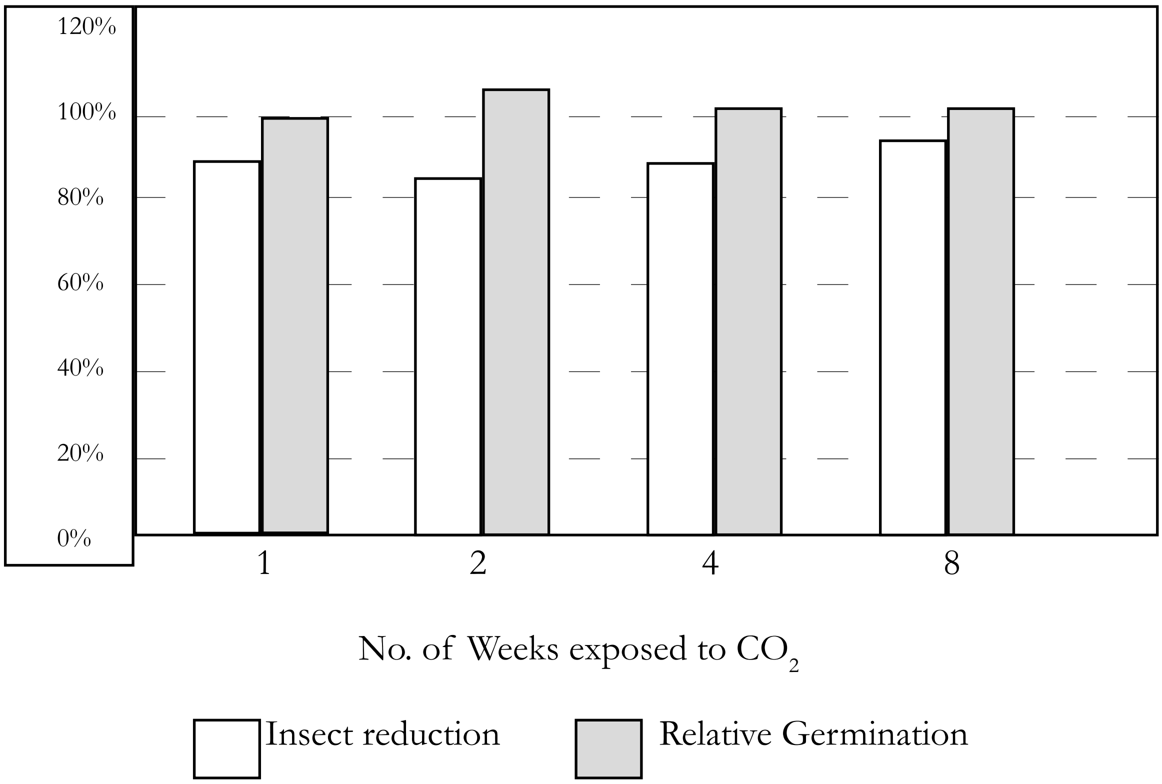


Figure 1. Insect mortality (adults, pupae, larvae and eggs) and seed germination in a series of 1 to 8 weeks' exposure in an atmosphere of initially around 60% CO_2 at 28°C. The seed was collected from stands of *Acacia* and *Bauhinia* in Burkina Faso, Africa.

2.3 Temperature

Probably all grain-infesting insect species can develop at temperatures between 15° and 40°C. Within this range the rate of development is highly affected by temperature. Outside this range, failure to hatch or emerge occurs (Navarro & Calderon 1980). The effect on insects of modified atmospheres (incl. CO_2) is much more pronounced at higher temperatures because of raised respiratory activity. Thus the effectiveness of CO_2 is reduced with decreasing temperatures. As the activity of the insects is high at temperatures of 20-29°C, this makes them more susceptible. The higher the temperature, the shorter the exposure period required to kill the insects. Very low temperatures (<0°C) or very high temperatures (>40-50°C) are in themselves lethal to insects. However, high temperatures may harm the seed.

2.4 Relative Air Humidity

A reduction of the relative humidity of the air increases insect mortality.

In very dry atmospheres (relative humidities about 20-25%) the mortality of adult insects was 100% after 5 days even when the concentration of CO_2 was as low as 4%. At relative air humidity of 59% to 97%, there was no significant effect at simlar CO_2 levels (Navarro & Calderon 1980).

Seed will attain an equilibrium moisture content with the relative humidity of the surrounding air. Only dry seed is storable in CO_2; for dry seed even small additional reductions in moisture content will decrease the relative humidity of the air in the storage bag. Reducing the moisture content of the seed from 8-12% to 5-7% will (depending on oil content) reduce the equilibrium relative humidity from around 60% to around 25% (Ellis *et al.* 1989). The conclusion is that the drier the seed the better is the effect of the CO_2 treatment.

2.5 Insect species, developmental stage and stress factors

The significance of insect species has been investigated closely for foodstuff and grain. But for Acacia seed many of the relevant insect species have not yet been taxonomically identified. Unfortunately, the species attacking seed of the Acacias seem to belong to the more persistent groups (DPIL/DFSC/CNSF, 1992).

The **developmental stage** of the insects is another decisive factor for the effect of CO_2 treatment.

Normally, the ranking is as follows

adult insects - large larvae - small larvae - eggs - pupae,

the adult insects being the most susceptible and pupae the most resistant ones.

Generally the adult insects also seem to be most susceptible to treatments like pesticides, freezing, vacuum etc. (Porsdal *et al.* 1989). However, exceptions occur.

The insect condition is significant. **Stress factors**, viz lack of food, drought, unfavourable temperatures, lack of oxygen, will generally interact to weaken the resistance of the insects.

3. EFFECT OF CO_2 ON SEED LONGEVITY

A number of studies indicate that CO_2 treatment of seed has no serious effect on seed longevity.

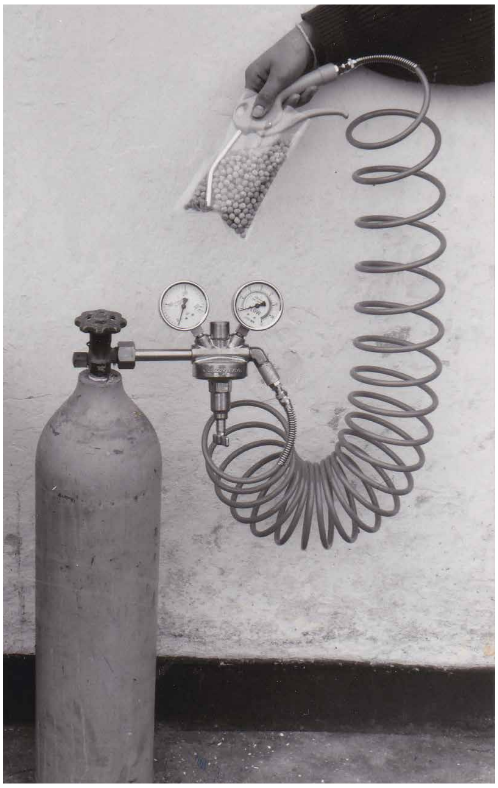
When respiring seeds are stored in sealed containers, the continuous reduction of the oxygen concentration gradually suffocates the seeds and reduces longevity. Moisture contents, based on fresh weight, must be below approximately 15% (depending on species) to be safe for hermetic storage (i.e. respiration close to nil) (Vertucci and Roos 1990). For long term storage, lower moisture contents should be aimed at.

In dry seed with moisture contents below 10% no detrimental effect of a CO_2 enriched atmosphere was detected for a number of species during 8 years of storage (Justice & Bass 1979), and this was confirmed in a number of studies of even longer duration. The viability of some *Acacia, Pinus* and *Prosopis* species and provenances stored in CO_2 at Danida Forest Seed Centre was not affected after 6 years' storage. Fungal and bacterial activitiy will most likely also be affected by the storage atmosphere and this indirectly affects seed viability (Moreno *et al.*, 1988; Suhargo, 1985). However, the effect of CO_2-enrichment on growth and development of these organisms in the seed is not well described.

At the present state of knowledge it seems safe to conclude that increased CO_2 concentrations do not reduce longevity of dried seed.

4. METHOD AND EQUIPMENT

Method



At DFSC the following method is used forCO$_2$ treatment. The dry seeds are placed in a laminated plastic bag with very low permeability to CO_2. The plastic bag is then kept upright with its opening as flat as possible while CO_2 is introduced through a tube into the bottom of the bag under the seeds (see figures 2 and 3). The CO_2, which is heavier than air, will flush out the air while filling the bag (just as if the bag was filled with water). Immediately after filling, the bag is sealed with a heat sealer. A vacuum will develop since CO_2 is absorbed by the seed. This method was described by Mitsuda *et al.* (1973); they called it the Carbon Dioxide Exchange Method.

The sealed bag is then placed at room temperature (approx 18^{o}C) for 8 weeks. At this temperature the insects are active and will be killed by the high CO_2 concentration. Subsequently the bag is transferred to the cold store for storage, or it is shipped to the seed user.

Equipment

Llaminated plastic bags and other possible containers

Of interest for CO_2 treatment in plastic bags is the permeability to CO_2 and water vapour of the plastic materials. Low permeability to water is mainly of interest during storage because the seed must be kept dry. The need of low permeability to CO_2 to keep the concentration high inside the bag is evident.

Ordinary plastic bags are made of low density polythene (LDPE). LDPE is heat-sealable but has high permeability to CO_2. Nylon (polyamide, PA) is not heat-sealable but has low permeability to CO_2.

Plastic bags made by laminating a layer incorporating nylon on the outside of an ordinary plastic bag combines the possibility of heat sealing with low CO_2 permeability. To prevent leakage from wear, the outer layer should be at least 30 µm thick (= 0.03 mm = 0.0012 inch). The seeds absorb CO_2 and this creates a vacuum (Mitsuda *et al.* 1973) as a result the bag collapses and tightly surrounds the seed. Consequently the inner LDPE layer should be strong enough to resist puncturing by the seeds and other sharp objects inside the bag. 70 µm (= 0.07 mm = 0.0028 inch) is usually enough.

Another suitable bag type is a laminate of aluminium foil and LDPE; it is strong and has a very low CO_2 transmission rate (see also Lauridsen *et al.*, 1992). Rigid containers such as cans cannot be used for CO_2 treatment as they would collapse or bend, because of the vacuum, to an extent where the gasket around the lid would not hold tight.

Laminated plastic bags described in the appendix have so far kept their low internal pressure for 6 years. The CO_2 concentration in bags containing *Pinus oocarpa* seed stored for at least three years at 4°C was 67% and the concentration of oxygen was 0.34 %, measured by gas chromatograph.

It is not advisable to use bags much larger than 4 litres as they tend to puncture easily.

CO_2 equipment

CO_2 is available in bottles in most places of the world. Small bottles (in Denmark containing 6 kg or less) are sold. Large bottles are rented.

The CO_2 in the bottle is liquid and at a pressure of approx. 40 atmospheres (bar) pressure. A 6 kg bottle contains 6 kg liquid CO_2. When the valve on the bottle is opened, the pressure inside the bottle falls and some CO_2 vaporizes. To reduce the pressure from 40 bar to the 1-3 bar possible in hoses etc., the bottle should be quipped with an adjustable pressure reduction valve. It is most convenient to use a flowmeter for CO_2. The flowmeter is adjusted to let a specified amount of vaporized CO_2 at 1 bar pass per minute.

If a CO_2 flowmeter is not available, a normal reduction valve for oxygen can be used (the threading on the bottles are the same in countries using British standard). An oxygen reduction valve should be able to produce a stable pressure of ½ to 1½ bar. The flowmeter or reduction valve can usually be procured at the company where refill bottles are purchased.

To fill CO_2 into the bags the flowmeter/reduction valve should be fitted with a hose ending with a valve (see fig. 2). The most convenient equipment is to use a hose and a blowing pistol for pressurized air. All connections between bottle, flowmeter/reduction valve, hose and blowing pistol should be tightly packed with tape in the same way as water pipes. Any CO_2 leaking from the joints can be found by applying a thin layer of soap water with a paint brush around the joints. The leaing CO_2 will blow bubbles in the soapy water.

Flushing with CO_2

The CO_2 should be let out at the bottom of the bag to be able to flush out the air. The speed of filling should be as fast as possible, to avoid mixing of air and CO_2, but without creating turbulence that will mix air and CO_2 even more and create air pockets between the seeds. 10-20 litres per minute would normally be reasonable for bag sizes up to 4 litres.

If a flowmeter is available, the litres per minute can be read immediately. When an oxygen reduction valve is used, a conversion factor between pressures in the hose and litres per minute will have to be established. The easiest way to do this is to:

1. collapse a big plastic bag inside e.g. a cardboard box of known volume,
2. adjust the reduction valve so that it has a pressure of e.g. 1 bar when the CO_2 flows freely through the valve at the end of the hose,
3. measure the time necessary to fill the bag so that it occupies the whole box.
4. divide the volume of the box by the time: e.g. 20 litres divided by 1½ minute = 13.3 litres per minute.

Experience shows that if the bag with seeds is filled with its own volume (the volume of the same bag sealed in completely expanded state), then the CO_2 percentage of the atmosphere is approximately 60%. Therefore, to have a safe margin, the bag is flushed with twice its own volume. As an example, a 4 litre bag should be flushed for 32 seconds if the flowmeter shows 15 litres per minute (15 l/min = 0.25 l/sec, 8 l ÷ 0.25 l/sec = 32 sec). CO_2 concentrations below 65% can be checked with Dräger gas detection tube equipment when the bag has been sealed (see appendix).

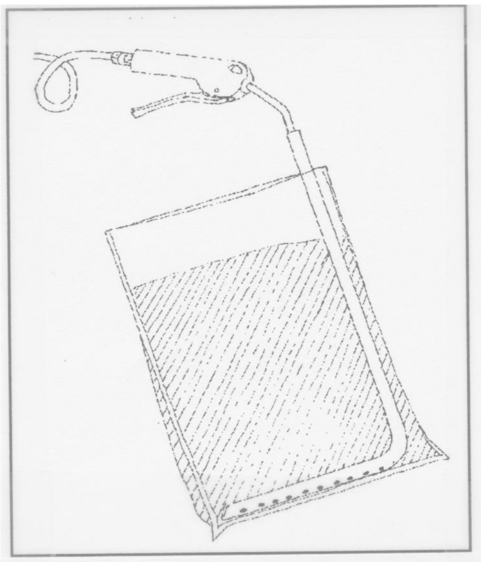


Figure 3. Tube for filling larger bags

Smaller bags can be filled by placing the tip of the blowing pistol at the bottom of the bag and moving it along the bottom sealing of the bag while blowing. For larger bags it is an advantage to construct a separate tube that will fit onto the end of the blowing pistol and along the bottom of the bag. It could, for example, be made from the brake fluid tube from a car. The bottom end should be plugged or squeezed tight. Small holes are drilled in the tube along the bottom of the bag so that the CO_2 is let out evenly. The tube illustrated in figure 3 is 7 mm internally and the drilled holes are 2 mm.

It is important that all sharp edges on blowing pistol, tubes, etc. are rounded and smooth so that they do not damage the bag.

Table 1 shows the gas concentrations measured by gas chromatograph bags were flushed with approximately three times their volume of CO_2. The pressure in the bag was reduced within 24 hours. The bags were stored for 0, 4 and 8 weeks, respectively, at 25°C. After 8 weeks the gas concentrations were still very suitable in regard to controlling/killing insects.

Storage weeks	0_2 %	N_2 %	CO_2 %	absolute pressure mm Hg
0	2.1	7.0	91.0	760
4	2.1	13.3	84.6	560
8	4.4	23.6	71.9	570

Table 1: Gas-concentrations in Riloten-X (40/70) bags stored at 25°C containing *Acacia nilotica* seed, measured by gas chromatograph at 0, 4 and 8 weeks, respectively, after CO_2 flushing.

It is interesting to note thaat a similar study conducted for *Pinus kesiya* seed showed lower concentrations of CO_2 (around 48%) in the bags, and after 4 weeks' storage the oxygen content was reduced to 1/20 of what it was immediately upon sealing the bag. This shows that the kind and amount of gases trapped in seed differ between species.

Sealing the bag

When the bag has been filled, it should be heat-sealed immediately. It should be kept upright until it has been sealed. The wall-mounted sealer quoted in the appendix allows this easily.

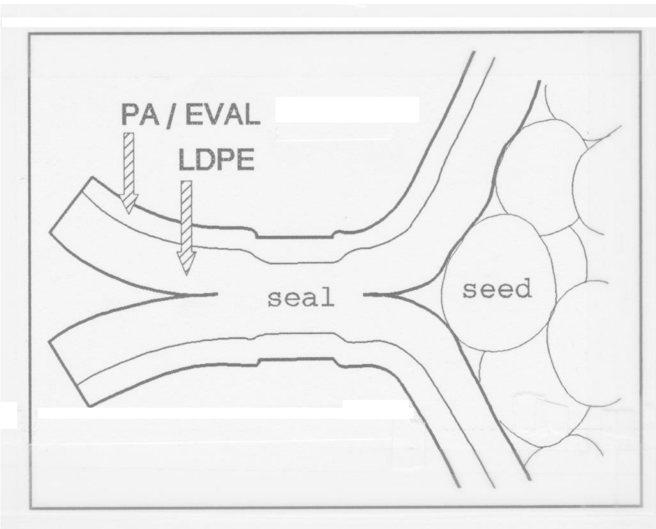


Figure 4. Cross-cut section of broad seal.

The seal should melt together a broad band of the two sides of the bag without melting any holes in the bag. This can only be done by heat sealers like the ones quoted here. where the temperature and sealing time can be adjusted and the sealing surface is broader than those made with a hot thread. It is also important to avoid any dirt or residues from the seeds being caught in the seal. It may be convenient not to fill the bag completely and leave space for two seals. In the space between the two seals the label for the seed lot can be placed. (fig. 6).

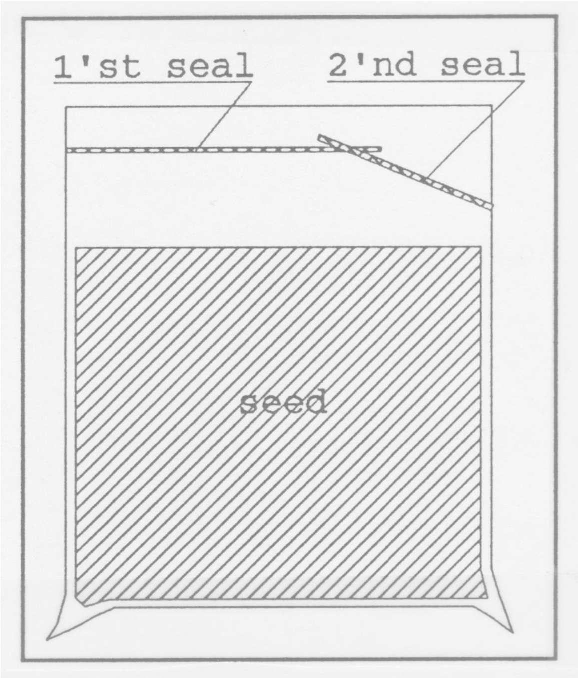


Figure 5. Large bag sealed in two stages

If the bag is broader than the heat-sealer (as shown in fig. 5(), a first seal should be made covering 3/4 of the top before flushing with CO2. After the flushing, a second seal stretching somewhat diagonally from the edge of the bag and across the first seal closing the bag. This also facilitates holding the opening of the bag flat during the flushing to prevent re-entry of air.

Handling the bag during treatment and storage

The outer CO_2-impermeable polyamid layer is very thin, so during the whole process it is very important to avoid scratching holes in the outer layer. Sliding the bag with seeds across a table with sand grains on will often puncture the outer layer.

Figure 6: CO_2 filled bags, labelled and sealed; the bags have tightened around the seed.

As the bag tightens around the seed (usually within 24 hours), it will attain a very solid state. It is therefore an advantage to pack the newly treated bags in square forms fitting the container in which they are going to be stored (and distributed) (fig. 6). Sharp creases in the bag should be avoided. As the bag will become solid and the plastic protrude somewhat over the seeds, the sides of the bag should be made as flat as possible to distribute the stress on the polyamid layer equally.

Lack of vacuum due to puncturing or inadequate treatment is easily detected during storage, this should be checked regularly.

Acknowledgement
The helpfulness of Mr. Peter Togeskov, O.N. Emballage, Lyngby, DK , in measuring atmosphere-compositions and pressure in bags is greatly appreciated.

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6. APPENDICES

6.1 Equipment appendix

Similar models to those mentioned here can often be found; it will be an advantage to find a local supplier for as much equipment as possible.

For each item the following is specified:

An 8-digit number, which is the database access-number of Danida Forest Seed Centre for the item (e.g. 400/06/404): the last number refers to the supplier. The name of the item. The name of the supplier. Specifications of the item. Price, currency and date of latest updating of the information. Remarks on the use and suitability of the item, optional equipment etc.

The item list is followed by a list of the addresses of the suppliers.

430/01/404: Laminated plastic bags for CO_2 packing (vacuum bags)

Specifications:	Riloten X 40/70, 0.5 - 4 litre bags
Price:	DKr 6600 per 5000 (92)
Supplier:	Slagteriernes Faellesindkoebsforening - See www.Danish Crown.dk

Remarks:

Co-extruded polyamide-6 (PA 6) and ethylene vinyl-alcohol (EVAL) adhesive-laminated to linear low density polythene (LLDPE). 0.04 mm PA 6 laminate/0.07 mm LLDPE.

WVR (Water Vapour Transmission Rate): < 4.5 g/m^2 24h.38^{o}C-90%. CO_2 transmission: < 8.0 cm^3/m^2 24h-1 atm-25^{o}C-75%RH (pressure in atm. refers to partial pressure of CO_2). The CO_2 transmission depends on the temperature; a rule of thumb is that for every 10^{o}C reduction of temperature the transmission is halved. The transmission also depends on relative air humidity. At relative air humidities below 30% the transmission is lowM at increasing relative humidities above 30% the transmission increases at an increasing rate.

Contact supplier for information on available sizes. Minimum order for production approx. 5000 bags. Laminated plastic bags are mostly used in the food industry for packing meat etc. One advantage of the laminate of PA/LDPE described here is that it is transparent.

The thickness of plastic bags is usually measured in mm, μ = 25 micron = 1 mil = 1 thou = 0.001 inch

NB! It should be noted that plastic bags laminated with nylon have approximately the same WVTR as ordinary DLPE bags of the same thickness. As the laminated bags are expensive compared to ordinary bags, they should be used only for CO_2 treatment and not for protection against moisture uptake during normal storage.

430/02/433 6 kg CO_2 bottle

Specifications: Not for rent. British standard threading.
Price: DKr 1464/pc (92)
Supplier: Otto Secher - Other suppliers available

Remarks:

British standard threading is Whitworth 21.8 mm diameter, 14 threads per inch, right-handed external thread. The standard is also used for oxygen bottles in many countries.

Larger bottles are usually rented.

Use a bottle that can be refilled locally.

A refill of a 6-litre bottle is 198 Dkr (92) at above dealer. See Figure 1.

Ask a workshop where they get oxygen and acetylene for welding. The oxygen company can also inform you on which types of bottles can be refilled locally.

430/03/433: Flowmeter AUTOGENA M44

Specifications: Flowmeter for carbon dioxide
0-32 litres per minute, British standard threading.
Price: DKr 619/pc (92)
Supplier: Otto Secher - Other suppliers available

Remarks:

Threading is usually the same as for oxygen bottles. If this is the case, a reduction valve for oxygen can be used although it does not indicate flow but only pressure. The oxygen reduction valve should be able to work stably at 1 to 1½ bar.

The reduction valve/flowmeter should be quipped with a hose and an air pistol for pressurized air. For filling of larger bags an extension of the pistol can be used. Flexible air hose DKr 142 (92), Air pistol DKr 47 (92).

430/04/434:	Gas detection pump with test tubes

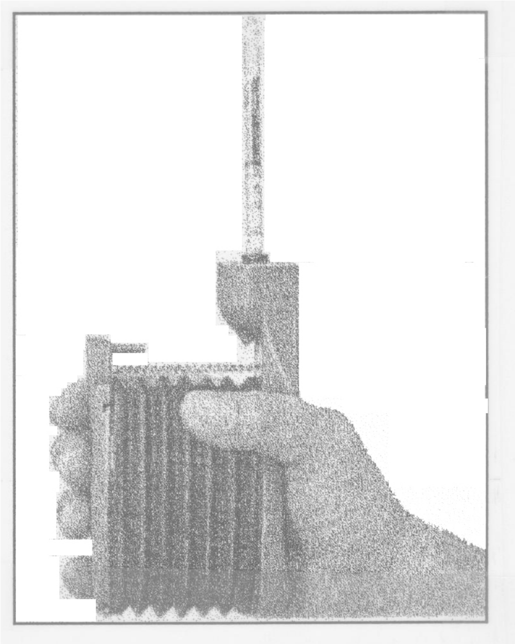
Specifications:	Gas detection pump in transport case with accessories
Price:	Dkr 2279/pc (92)
Supplier:	Dräger Teknik a/s

Remarks:

The pump sucks air through a glass tube containing indicating chemical.

Tubes for CO_2 testing bought separately: Cat. no. CH 20301 Carbondioxide 5-60% volume, 10 pc, Dkr 354 (92).

Tubes for a wide variety of gasses available.



451/03/003: Plastic Bag Sealer

Specifications: Cat. no. 580SA: SUPER SEALBOY Bench mount with foot pedal 115V 60Hz, 23" seal bar, incl. knife

Price: US$ 1075/pc (91)

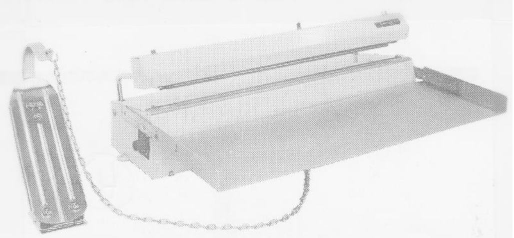
Supplier: Seedburo Equipment Company

Remarks:

Note power specifications,
Cat.no. K20 Transformer for 220 V
US$ 137 (91)

Cat.no. 580T optional feed tray
US$ 84 (91)

Can also be bought for 220V from dealer 407 Genpack as AUDION SEALMASTER A SSM 580 SA for Dkr 7425 (91)



No. 580SA with No. 580T (optional tray)

451/04/407: Plastic Bag Sealer

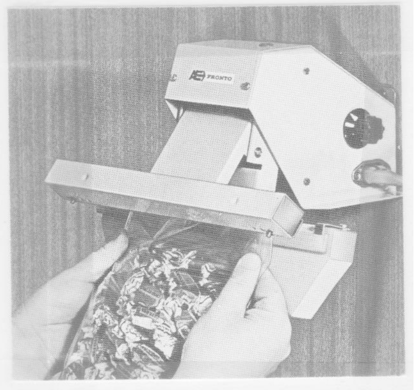
Specifications: Cat.no.A PR 255 A: AUDION PRONTO wall/table mounted, 220V, 255 mm seal bar, timer

Price: Dkr 2765/pc (91)

Supplier: Genpack

Remarks:

The construction of the sealer allows sealing without spilling seed. Delivers a broad effective seal. Cat.no. A PR 420 A is similar, but with 420 mm seal bar at Dkr 3740 (91).



6.2 Suppliers (updated October 2014)

003	Seedburo Equipment Company 2293 S Mt Prospect Rd Des Plaines IL 60018, United States	Int. Phone: +1 800-284-5779 www.globalmilling.com/seedburo-equipment-company
407	Genpack Hirsemarken 6 DK-3520 Farum Denmark	Int. Phone: +45 44 48 62 00 www.genpack.com
434	Dræger Teknik a/s Generatorvej 6 B DK-2730 Herlev Denmark	Int. Phone: +45 50 00 00 00 www.draeger.com